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FOREST INSECT INVESTIGATIONS

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# CRITICAL LOW TEMPERATURES FOR THE WESTERN PINE AND MOUNTAIN PINE BEETLES

## SECOND PROGRESS REPORT OF LABORATORY STUDIES

1933-1934

By

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# INTRODUCTION

A review of experiments dealing with critical low temperatures affecting barkbeetles was presented in a report by Miller and Struble in February, 1935. This report included the results of past and recent laboratory work that had been completed up to January, 1935.

Results of these studies show very positive differences in cold resistance between the western pine beetle and the mountain pine beetle. It was shown that for the overwintering larvae of these two species, critical temperatures for the western pine beetle ranged from +5 to -7.5° F., while those for the mountain pine beetle ranged all the way from +14 to -20° F. It was also found that mortality of the western pine beetle occurred consistently whenever bark temperatures were chilled down to the critical range and that this range was the same for material from all regions and all environmental conditions. On the other hand, mortality of the mountain pine beetle varied according to host, region and condition of seasonal development, so that the material collected under one particular set of environmental conditions may have an entirely different range of critical temperatures from material collected under other conditions.

From the results of the experiments with these two species, it was concluded that in dealing with the problem of winter kill in the field, the critical range for the western pine beetle had been sufficiently well established to determine from weather records when mortality may be expected. With the mountain pine beetle, however, much further work must be done before the factors which produce winter kill are definitely understood.

It was also pointed out that tests with the egg and first instar larval stages of the western pine beetle had been unsatisfactory, due to the high mortality from other causes than when handling material in these stages in refrigeration experiments.

The relation of time of exposure to mortality was given some attention in these studies, and it was shown that with the advanced larval broods of the western pine beetle mortality increased with time at the intermediate critical temperatures, but that it became 100% complete soon after the larvae were chilled to the lowest poine in the critical range. The need for further verification of these results and study of the time factor was indicated.

Preliminary tests indicated that summer brood larvae of the western pine beetle are less resistant, the mortality being higher at the upper points in the critical range, than with larvae of the winter broods. Certain experiments indicated that the resistance of these active summer brood larvae may be slightly increased by conditioning prior to exposure to critical temperatures. This conditioning was accomplished in the laboratory by alternating the infested bark between warm and cold temperatures of 70° and 30° F. The preparation of broods during the fall period appears to be a factor influencing cold hardiness that may be of

considerable importance to the winter kill of barkbeetles, and is a field of study which warrants further experimentation.

Since January, 1935, experiments have been continued at the Berkeley laboratory mainly with the object of supplementing and continuing the studies presented in the first progress report. In this supplemental work extending from January to May, 1935, special attention was given to the following points:

A. Rechecking of the more doubtful results secured in the Western pine beetle studies, particularly those pertaining to (1) conditions which may increase or lower cold resistance, (2) relation of time of exposure and brood stage to mortality, and (3) effect of critical temperatures upon subsequent development of surviving larvae.

B. Continuation of all phases of mountain pine beetle studies, including relation of critical temperatures to host, region, brood stages and seasonal activity of broods.

This work was carried on under the same cooperative arrangements and with the equipment described in the progress report of February, 1935. The terms here used are the same as those previously defined.

I. General Methods:

Bark removed from infested trees was brought to Berkeley by truck and, unless otherwise noted, was stored out of doors in the shade until needed. In all tests, except one requiring eggs and small larvae, the brood was removed from the bark and placed in paraffin cells molded in petri dishes, as described in the first report. The larvae were then chilled at 36° F. before being transferred to the experimental refrigerator unit. The purpose of this pre-chilling is to prevent any possible effect of extreme sudden shock, which might occur if transfer were made directly from room temperature to points within the critical range. After exposure, the larvae were removed to an incubator in which the temperature was about 70° F. and 65% humidity in order to observe development of survivors. Observations were made at 24 and 48 hour and 10 day intervals.

The most important change in the procedure was in the operation of the laboratory refrigerator unit. Previously it had been the custom either to shut off the machine at night or to set the thermostat at a given point, and leave the material being tested at that temperature over night. It was decided that such a practice might result in complications due to the effect of the time factor. Accordingly, in the tests here reported each was carried to completion without interruption.

II. One Effect of Environmental Temperatures on the Critical Range.

It had been determined previously that cold hardiness is greater in the winter than the summer broods. The causes of this change were, however, not known. The following tests were performed in an effort to obtain more information on this problem.

Experiment No. 18
Feb. 13 and 14, 1935

Object: To determine the lower intermediate and ultimate critical points of brood in midwinter.

Material and procedure: Host, Pinus ponderosa. Bark collected Feb. 2, 1935, in Modoc National Forest. Mature larvae removed from bark Feb. 12 and placed at 36° for chilling; transferred to laboratory refrigeration machine (at 10° F.) 2:00 P.M. Feb. 13.

Results:

TABLE 27

| Sample No. | Degrees F.: Temperature | No. Larvae | % Mortality 48 hrs. after exposure |
|---|---|---|---|
| Check | 36 | 50 | 0 |
| 1 | 5 | " | 16 |
| *2 | 0 | 46 | 50 |
| *3 | 0 | 50 | 42 |
| 4 | -5 | " | 96 |
| 5 | -7½ | " | 100 |

Discussion: In comparing these results with those of earlier experiments, it will be noted that although there was considerable variation in the mortality at intermediate critical points, the ultimate critical temperatures were in every case nearly the same. This would seem to indicate that there is little change in cold hardiness of winter brood larvae as the season progresses.

Experiment No. 19
Jan. 25 to Feb. 4, 1935

Object: To determine the effects on cold hardiness that may be induced by subjecting larvae of winter broods to developmental and subdevelopmental temperatures, i.e., active as opposed to inactive larvae of Exp. 18.

Material and procedure: Host, *Pinus ponderosa*. Bark collected in Lassen National Forest Dec. 10, 1934. Two lots of bark made up; Lot "a" placed at 70° and lot "b" placed at 36°. After 1 week the mature larvae were removed from the bark and placed at 36° over night for chilling, and transferred to the laboratory refrigeration unit (at 12° F.) the following morning for exposure to temperatures within the critical range.

Results:

TABLE 28

| Sample No. 50 each | Temperature: Degrees F. | Mortality a | Mortality b |
|---|---|---|---|
| Check | 36 | 0 | 0 |
| 1 | 7½ | 10 | 2 |
| 2 | 5 | 18 | 8 |
| 3 | 2½ | 32 | 22 |
| 4 | 0 | 46 | 30 |
| 5 | -2½ | 66 | 22 |
| 6 | -5 | +100 | 100 |
| 7 | -7½ | 100 | 100 |

- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

* Placed in 0° room of Hilgard Hall for 8 hours
\+ Refrigerator shut off during night 5½ hours

Discussion: A pronounced difference in cold resistance appears in the middle critical range, but the ultimate critical point is the same for both lots. In comparing with data from previous tests (Fig. 1), it will be observed that the mortality curve for the larvae held at 36° (lot b) is a little below the average mortality curve for inactive winter brood, whereas the curve for developing larvae (lot a), while somewhat higher, is decidedly below that for summer brood. It seems strange that, although it is possible to alter the intermediate critical points for both summer and winter broods, we have as yet been unable to discover conditions which will cause any appreciable change in the ultimate critical points. This means essentially that the resistance of most of the individuals can be altered by altering conditions, but in some it seems cold resistance remains constant. Whether this may be due to some inherent characteristics of the species (genetic?) or to some undiscovered environmental factor, we do not know at present.

## III. Time of Exposure Necessary for Critical Temperatures to Produce Mortality

Because of the variation in the results which were presented in the first report, it was thought desirable to repeat some of the tests.

### Experiment No. 20, Jan. 19 and 20, 1935

Object: To make further comparison of the effects on winter brood of sudden and gradual exposures to points within the critical range.

Material and Procedure: From Ponderosa pine bark collected Dec. 10, 1934, in Lassen National Forest, mature larvae taken from bark Jan. 16 to 18, and placed at 36° as removed. The same methods of exposure employed as in experiment 5b (Page 12) of the previous report; lot "a" transferred to the laboratory refrigerator (at 10° F.) 8:40 a.m. Jan 19 and units removed after stabilizing temperature at the desired points. Units of lot "b" transferred to the machine when the latter was stabilized at the desired temperature.

Results:

TABLE 29

| Lot | 0° No. larvae | 0° % mortality | -2° No. larvae | -2° % mortality | -4° No. larvae | -4° % mortality | -5° No. larvae | -5° % morty | Check No. L | Check % mortality |
|---|---|---|---|---|---|---|---|---|---|---|
| 20a | 49 | 32.2 | 48 | 22.9 | 49 | 51.0 | 50 | 100.0 | 49 | 0 |
| 20b | 49 | 18.3 | 49 | 46.9 | 48 | 76.6 | 50 | 100.0 | | |

Discussion: As in Experiment 5, there is such variation in the results that no conclusion can be drawn. If, however, the variations in mortality in these tests involving sudden vs. gradual exposure be due to individual variation in the larvae used, then a more reliable expression of the data should be obtained by employing averages. When this is done, it appears that the mortality rate for larvae subjected to sudden exposure is

is definitely less for the intermediate critical points and would suggest that the difference is due to the time factor involved in the gradual exposure, and that sudden shock in changing quickly to a lower temperature has no additional effect. These data are much too inadequate a basis for any but tentative conclusions.

Experiment No. 21,
March 14, 1935

Object: To re-determine the effect on larvae of different lengths of exposure to points within the critical range.

Materials and procedure: Ponderosa pine bark collected Feb. 2, 1935. Stored out of doors in Berkeley until Mar. 1, when it was placed at 41° to prevent the larvae from becoming active. Mature larvae removed from the bark Mar. 12 and 13; removed to 36° room for chilling. Each dish was placed individually in the laboratory refrigerator, which had been stabilized at the desired temperature. Ten minutes (determined in previous tests) was allowed for the container to cool to the temperature of the compartment and the desired period of exposure counted after this period of adjustment.

Results:

TABLE 30

| Sample No. | No. Larvae | Temperature Degrees F. | Period of Exposure | % Mortality (48 hours after exp.) |
|---|---|---|---|---|
| 1 | 50 | -7.5— -8.0 | 15 min. | 100 |
| 2 | " | -7.5— -7.8 | 30 " | 100 |
| 3 | " | -7.4— -7.8 | 50 " | 100 |
| 4 | "" | -5.0— -5.7 | 15 " | 100 |
| 5 | " | -4.7— -5.3 | 30 " | 100 |
| 6 | " | -4.8— -5.1 | 45 " | 100 |
| 7 | " | -2.2— -2.6 | 15 " | 62 |
| 8 | " | -2.3— -2.7 | 30 " | 48 |
| 9 | " | -2.3— -2.6 | 45 " | 36 |
| 10 | " | -2.2— -2.7 | 60 " | 54 |
| Check | " | 36° | -- | 0 |

Discussion: A comparison of these results with those given in Experiment 3 shows somewhat less mortality in Exp. 3 at -2½°. The striking thing, however, is that after a variable mortality over the range of exposure at -2½, at -5° and -7½°, the mortality abruptly rises to 100%, even for the shortest exposures. This is at variance with the results obtained in Experiment 3,- perhaps because the temperature control at that time was not accurate. The cause of this sudden rise in mortality between -2½° and -5° is not known. It may be that the mortality at -2½° is the resultant of low temperature and other factors, whereas at -5° low temperature alone is the limiting factor. Further tests on this phase would be desirable.

## IV. Relation of Brood Stages to Mortality Resulting from critical Temperatures.

The results of Experiments 6, 7 and 8 give us information on the critical range of 4th instar larvae, pupae and adults, but none on eggs and small larvae. Tests on the latter had not been made because of the difficulty of securing material and handling it during the tests. This spring, however, an attempt was made to develop a technique for securing determinations.

### Experiment No. 22
### March 8, 1935

Green ponderosa pine logs were artificially infested in the laboratory and material secured after allowing sufficient time for oviposition and hatching. The eggs and small larvae (less than 1/4 grown) were placed in paraffin cells; a piece of wet paper toweling placed in each petri dish to prevent drying. However, this precaution was not adequate, as it was found after the exposures had been made that practically all the eggs and larvae had died due to desiccation.

A second attempt was made to study the effects of temperatures on eggs and small larvae; this time by exposure to one temperature only, -0°. The tests were conducted by G. R. Struble.

### Experiment No. 22a.
### March 28 and 29, 1935

Object: To determine the mortality to eggs and small larvae *in situ*, resulting from 25 hours exposure to a temperature of 0°F.

Material: One of the logs mentioned in Experiment 22 was cut in two equal parts; one part was placed in the 0° room in Hilgard Hall and the other was placed in the 36° room. After 24 hours that in the 0° room was transferred to the 36° room where both lots remained until mortality analyses were made April 2, 1935.

Results:

TABLE 31

| | Larvae | | | Eggs | | |
|---|---|---|---|---|---|---|
| Temperature | Alive | Dead | % Mortality | *Alive | Dead | % Mortality |
| 0° | 246 | 82 | 25 | 128 | 32 | 20. |
| 36° check | 200 | 0 | 0 | 114 | 54 | 33.8 |

*Determined by the number hatching following the exposure.

As a supplemental test, 16 of the larvae which were removed during the bark analyses were placed in a petri dish on moist blotting paper and exposed to 0° for 15½ hours. All the larvae died.

Experiment 23b,
April 2 and 3, 1935

Object: To compare the mortality of 4th instar larvae in bark with the results of the above test.

Material and procedure: Infested bark collected at Sierra National Forest March 19, 1935, brought to Berkeley and placed in 36° room April 2. One lot was brought to room temperature for 18 hours to induce activity in the brood and was then placed in the 0° room 24 hours. Following exposure the bark was placed in the 36° room until analysis was made on April 5.

Results:

| | Larvae | Pupae |
|---|---|---|
| Alive | 4 | 0 |
| Dead | 85 | 1 |

Discussion: We know, from the studies on bark temperatures reported by Beal (1), that there is a considerable difference between subcortical and air temperatures. This is evidently the reason for low mortality in the eggs and small larvae in Experiment 20a. The fact that the naked larvae all succumbed and that the mature larvae in dry bark (20b) had such a high mortality substantiates this viewpoint. Further tests using an improved technique will be necessary for reliable results.

V. Effect of Exposure to temperatures within the critical range on subsequent development of survivors.

The results of all previous tests, except those for prolonged exposures at 0°, have been based upon mortality found 48 hours after exposure, with no regard to the effect on later development of survivors or subsequent mortality resulting from cold shock. This is an important point, as the ultimate mortality may be somewhat higher than that indicated on first examination after a freeze.

With this in mind, the surviving material of a number of previous tests has been kept in rearing. This material is largely from Experiments 4a, 4b, 4c, 5a, 5b, 18, 19 and 20, described in the first progress report. Tables of results of subsequent rearings have been worked up but are omitted from this report because of their length and the fact that they show no positive correlations.

There appears to be no correlation between ~~temperature and~~ development of survivors. Between length of exposure at 0° and development of survivors, no significant correlation was apparent until a period of 3 or 4 days had been covered. Following these long exposures, the surviving larvae lose the ability to pupate. From the great variations found in tests at other points, one is lead to believe that other variables are involved.

## VI. Freezing Point of mature Larvae.

Experiment No. 24
Feb. 21, 1935

Object: To determine the freezing point of the free (?) water in the bodies of mature larvae.

Material and procedure: Infested bark collected in Modoc National Forest Feb. 20, 1935; mature larvae removed from bark Feb. 20 and placed at 36° for chilling. The refrigerator was stabilized at the desired temperature and one lot of larvae introduced and exposed for 1½ hours. After exposure the larvae were examined immediately by touching with a camel's hair brush to determine whether or not they were frozen.

Results: In this test we were unable to duplicate the previous work in which it had been found that larvae would freeze at 12° to 14° and that on thawing out the frozen larvae appeared perfectly normal. In these tests it was found that the larvae did not freeze until exposed to a temperature of 10° or less and that the effects of freezing in practically every case were fatal.

Discussion: To arrive at any definite conclusions on this matter, further tests would be required and quite possibly would lead into a study of bound water, a field in which adequate technique has not as yet been developed and opinions are highly controversial.

## VII. General Discussion on D. brevicomis experiments.

The results presented above will add little new information to that contained in the first report, as most of the tests were performed as checks against the previous work. Two very striking results, however, are brought out. First, the exposure of larvae to decreasing critical temperatures, either by gradual or sudden exposure, results in an apparently random mortality until a point close to the ultimate critical point is reached. At this point the mortality rate suddenly rises and at the next lower interval reaches 100 percent. When averages are plotted a curve indicating a rising mortality rate with a decreasing temperature is indicated, but there are not enough data to definitely establish such a curve. The development of survivors also appears to be random, evidently bearing no relation to the temperature of the exposure nor the rate of mortality. Such results, as mentioned above, lead one to believe that either we are dealing with an inherently extremely heterogeneous species, which seems rather doubtful, or some of the larvae have been pre-conditioned by environmental factors, either in the field after collection or during the exposures. There are two avenues of approach to this problem: (1) if it were possible to establish a field laboratory for making the exposures, the factor of transportation and storage following collection would be eliminated, and, (2), if a satisfactory synthetic diet could be worked out for *D. brevicomis*, the factor of nutrition difference in different

spots in the bark could be eliminated. Since it is known that in some insects there is a definite relationship between fat, nutrition and cold hardiness, it might even be desirable to make determination of the fat content of the larvae. Furthermore, it will be exceedingly desirable to follow brood from different localities through an entire yearly cycle to determine more accurately and completely the time and extent of seasonal changes in cold hardiness, and to conduct tests on the effect of different lengths of exposure to various critical points in order to make possible a closer application to winter field conditions.

## MOUNTAIN PINE BEETLE STUDIES

### I. General Methods:

Brood from the field was brought to Berkeley in 3 foot logs and, if the weather was cool, stored out of doors until needed; otherwise they were stored in the 36° room in Hilgard Hall. When making tests the larvae were removed from the logs and placed in paraffin cells in petri dishes. In the earlier experiments the larvae were placed in the 36° room overnight for chilling, but this step was eliminated in the later tests because it was felt that exposures should be made as soon after the larvae were removed from the bark as possible, to reduce mortality due to desiccation.

### II. Critical Temperature in Relation to Host.

In the preceding tests an effort was made to compare the cold hardiness of D. monticolae larvae from different hosts, but since larvae from different hosts had all come from different localities such comparison could not be reliable. This spring an effort has been made to secure larvae from the different hosts in the same locality.

Critical Range in Sugar pine from Central Sierra Region.
Experiment No. 25
January, 1935

**Object:** To determine the critical range for larvae from sugar pine in order to compare with similar data on larvae from ponderosa pine and to determine any change in the critical range of winter brood during the winter season.

Experiment No. 25a
Jan. 2, 1935

**Material and procedure:** Larvae from forced attack made at Sierra National Forest Sept. 21 to 25, 1934; brought to Berkeley and stored out of doors until Dec. 15, when they were transferred to 36° room to prevent pupation. Removed from bark Jan. 2, 1935, and placed in 36° room over night.

Results:

| Number | Temperature | 1/2 to full grown: No. larvae in sample | 1/2 to full grown: % mortality 24 hrs. after exposure | 1/3 grown: No. of larvae in sample | 1/3 grown: % mortality 24 hrs. after exposure |
|---|---|---|---|---|---|
| Check | 36° | 100 | 0 | 49 | 24.5 |
| 1 | 15 | 50 | 2 | 50 | 20 |
| 2 | 12½ | " | 26 | " | 44 |
| 3 | 10 | " | 66 | 44 | 57.5 |
| 4 | 7½ | " | 78 | 50 | 92 |
| 5 | 5 | " | 100 | " | 98 |

Experiment 25b
Jan. 24, 1935

Material and procedure: Same as 25a; removed from bark Jan. 23, 1935.

Results:

| | | Mature | | half-grown | |
|---|---|---|---|---|---|
| Check | 36° | 50 | 4 | 0 | 0 |
| 1 | 15 | 0 | 0 | 50 | 6 |
| 2 | 12½ | 50 | 52 | 0 | 0 |
| 3 | 10 | 0 | 0 | 50 | 50 |
| 4 | 7½ | 50 | 58 | 0 | 0 |
| 5 | 5 | 41 | 63.4 | 50 | 94 |

Experiment 25c
March 19, 1935

Material and procedure: Infested logs from Yosemite National Park brot to Berkeley March 19, removed from bark at once, placed in laboratory refrigerator at 50°.

Results:

| Number | Temperature Degrees F. | Number of larvae in sample | Percent Mortality 2 to 12 hours after exposure |
|---|---|---|---|
| Check | 70 | 50 | [illegible] |
| 1 | 15 | " | 0 |
| 2 | 12½ | " | 46 |
| 3 | 10 | " | 68 |
| 4 | 7½ | " | 80 |
| 5 | 5 | " | 60 |
| 6 | 2½ | " | [illegible] |
| 7 | 0 | " | [illegible] |

Experiment 25d
April 9, 1935

Material and procedure: Infested logs from Yosemite National Park brought to Berkeley March 19; stored at 36° and removed from bark April 9. Placed in laboratory refrigerator at 30°.

Results:

| Number | Temperature | Half-grown to Mature Larvae: Number larvae in sample | Half-grown to Mature Larvae: % mortality 1 to 12 hrs. after exposure | Less than Half-grown: Number larvae in sample | Less than Half-grown: % mortality 1-12 hours after exp. |
|---|---|---|---|---|---|
| Check | 70 | 25 | 0 | 25 | 0 |
| 1 | 12½ | " | 4 | " | 28 |
| 2 | 10 | " | 20 | " | 44 |
| 3 | 7½ | " | 48 | " | 60 |
| 5 | 5 | " | 64 | " | 76 |
| 6 | 2½ | " | 92 | " | 92 |
| 7 | 0 | " | 96 | " | 100 |

Discussion: The larvae used in 25a had been subjected to so many different conditions that one could not say with any degree of certainty that they represent summer brood. However, if we assume that they dom then it is evident that there has been a considerable degree of hardening with the advent of winter conditions. There is some difference in mortality rates between mature and small larvae, but the higher rate in the latter may be due to other conditions. There is considerable difference in the degree of cold-hardiness in the above tests and those given in the first report. This may be an inherent difference in brood or quite possibly may be due to difference in handling and inaccuracies in temperature control.

Critical Range in Ponderosa pine from Central Sierra Region
Experiment No. 26
May 6, 1935

Object: To determine mortality at critical points for larvae from ponderosa pine from middle Sierra region.

Material and procedure: Infested logs brought into Berkeley May 4; part stored out of doors and part stored at 36°. Both lots removed from bark, sorted for size and placed in laboratory refrigerator at 31° May 6, 1935.

Results: a- from material kept out of doors; b-that stored at 36°

| Number | Temperature °F. | No. larvae in sample | % mortality 8 hrs. after exposure | Number of larvae in sample | % Mortality 8 hours after exposure |
|---|---|---|---|---|---|
| a | 5 | 50 | 96 | 32 | 67.5 |
| b | 2½ | 49 | 91.8 | 45 | 97.8 |

Discussion: It was very disappointing that we were unable to obtain more larvae for this test. It had been hoped that the results would be a star in settling a number of points concerned with host differences. It does appear however, that these larvae have a mortality rate somewhere near that of the larvae of sugar pine in the same locality, and may indicate that, other factors being equal, the matter of host selection bears no relation to cold-hardiness. Of course, it is realized that, assuming these limited data are accurate, the larvae may have resumed activity in the field and, consequently, had a lesser degree of cold resistance than they had in midwinter.

## III. Critical temperatures in relation to locality.

Critical temperatures in Ponderosa pine from northeastern California in comparison with that from Central Sierras.
Experiment No. 27
March 26 and 27, 1935

Object: To determine the critical range for larvae in Ponderosa pine from the northeastern California region for comparison with brood from the same host from the Central Sierra region.

Material and procedure: Infested logs collected in Modoc National Forest and brought to Berkeley March 25; placed in 36° room over night; 1/2 to full grown larvae removed from bark March 26 and placed in laboratory refrigerator at 50°.

Results:

| Number | Degrees F. Temperature | No. of larvae in sample | Percent mortality 2 to 17 hours after exposure |
|---|---|---|---|
| Check | 70 | 46 | 0 |
| 1 | 15 | 49 | 0 |
| 2 | 12½ | 50 | 0 |
| 3 | 10 | 48 | 0 |
| 4 | 7½ | 50 | 0 |
| 5 | 5 | 49 | 0 |
| 6 | 2½ | 50 | 6 |
| 7 | 0 | " | 6 |
| 8 | -2½ | 49 | 18.4 |
| 9 | -5 | 48 | 41.7 |
| 10 | -7½ | 50 | 90 |
| 11 | -10 | " | 96 |

Discussion: The test points very definitely to a much greater cold resistance for larvae from the colder northern region than for those from the warmer southern area. Perhaps even more surprising is the apparently greater resistance of the northern D. monticolae than that of D. brevicomis from the same host in this same northern California region. Further tests on regional differences should prove very enlightening.

## IV. Critical temperatures in relation to activity and stage of development.

The results presented in the first report indicate that there is a difference in resistance between brood in different stages of development and between active and inactive larvae of the same stage of development. The following tests were made as a check on these results:

Critical points for larvae in sugar pine when subjected to developmental temperatures and to alternating developmental and sub-developmental temperatures.
Experiment No. 28
March 29, to April 9, 1935

Object: To determine the critical points for overwintering larvae which have been subjected to developmental temperature and to alternating developmental and sub-developmental temperatures.

Material and procedure: Infested sugar pine logs from Yosemite National Park brought to Berkeley March 19. One lot, "a", placed at 70° for ten days before exposures were made; lot "b" was subjected to alternating periods of 8 hours at 70° and 16 hours at 32° for 12 days; lot "c" were subjected to alternating periods of 8 hours at 70° and 16 hours at 20° for 17 days.

Results:

| | | -a- | | -b- | | -c- | |
|---|---|---|---|---|---|---|---|
| Number | Temperature Degrees F | No. larvae in sample | % mortality 4/17 hrs. after exposure | No. larvae in sample | % mortality 1/10 hrs. after exposure | No. larvae in sample | % mortality 2/14 hrs. after exposure |
| Check | | | | 19 | 0 | 25 | 0 |
| 1 | 17½ | 25 | 4 | | | | |
| 2 | 15 | 50 | 2 | | | | |
| 3 | 14 | " | 28 | | | | |
| 4 | 12½ | " | 56 | 50 | 16 | 25 | 44 |
| 5 | 10 | " | 96 | 49 | 24.5 | " | 56 |
| 6 | 7½ | " | 100 | 50 | 72 | " | 84 |
| 7 | 5 | 25 | 100 | " | 94 | " | 96 |
| 8 | 2½ | | | " | 100 | " | 96 |
| 9 | 0 | | | " | 100 | " | 100 |

Discussion: As in the previous tests, we find that active larvae are much less resistant to low temperatures than are the the inactive overwintering brood, and seems to bear an inverse relation to the environmental temperature; for example, the larvae which were subjected to the alternating exposures are less resistant than those carried directly from the overwintering conditions in the field and those kept at 36°. On the other hand those from the alternating exposures are much more resistant than those kept at 70°. The difference in the

ultimate critical points between these and the former tests are probably due to improved temperature control and methods of handling larvae. These results suggest interesting possibilities for future tests.

Comparison of critical ranges for mature larvae and pupae.
Experiment No. 29-
<u>March 29, "a" and April 18, 1935, "b"</u>

Object: To compare the critical range of pupae with that of mature larvae, in order to determine the effect of stage of development upon resistance to low temperature.

Material: Infested sugar pine logs from Yosemite brought to Berkeley and kept at room temperature until mature larvae pupated. Larvae and pupae removed and placed in laboratory refrigerator at 25°.

Results:

| | | "a" | | "b" | | "c" | |
|---|---|---|---|---|---|---|---|
| Number | Temperature Deg.F. | No. larvae in sample | % mortality 4/17 hrs. after exposure | No. pupae in sample | % mortality 4/17 hrs. after exposure | No. pupae in sample | % mortality 9 to 11 hrs. after exposure |
| 1 | 17½ | 25 | 4 | 25 | 8 | | |
| 2 | 15 | 50 | 2 | 50 | 4 | | |
| 3 | 14 | " | 28 | " | 0 | | |
| 4 | 12½ | " | 56 | " | 4 | | |
| 5 | 10 | " | 96 | " | 14 | | |
| 6 | 7½ | " | 100 | 40 | 50 | | |
| 7 | 5 | 25 | 100 | 25 | 32 | | |
| 8 | 2½ | | | | | 9 | 5.6 |
| 9 | 0 | | | | | 49 | 93.8 |
| 10 | -2½ | | | | | 25 | 100. |
| 8 | 2½ | clerid larvae | | | | 31 | 100 |

Discussion: The difference between active larvae and developing pupae is quite pronounced in both the above and preceding experiments. Just why this difference should exist is not evident. However, we do know that cold resistance and hibernation are associated with a reduction of water content, and we also know that transformation from the larval to the pupal stage may also be accompanied by a decrease in water content. It may be, then, that the loss of water during pupation increases cold hardiness in a purely incidental manner. Tests on pupae of summer and fall broods should throw more light on this point.

As an interesting sidelight, the clerid larvae tested were all dead at 2½° while pupae survived. This, at times, might be a factor in natural control.

V. General Discussion of D. monticolae experiments.

As in the tests on the western pine beetle, the information we have at present points the way to a number of possibilities, but considerable more work is needed to make these low temperature studies at all complete. A further investigation of the effect of host difference and locality difference particularly would be desirable, as well as comparative seasonal studies. It should also be desirable to develop a technique for testing eggs, young larvae and subsequent development of exposed larvae and pupae. To date all attempts have resulted in a high mortality from desiccation. Again, as in the D. brevicomis tests, the matter of changes in the material during transportation to Berkeley is involved and, if it were possible to make at least some tests in the field, we could have a much better idea just what factors we are dealing with.

REFERENCES


(1) 1934, Beal, J. A. — Relation of Air and Bark Temperatures on Infested ponderosa pines during subzero weather. Journ. Ec. Ent. Vol. 17, pp. 1133- 1139.

(2) Miller, J.M. and Struble, G.R. — Critical Low Temperatures for western pine and mountain pine beetles. Progress Report of Laboratory Studies 1933-1934.

FIG. 1

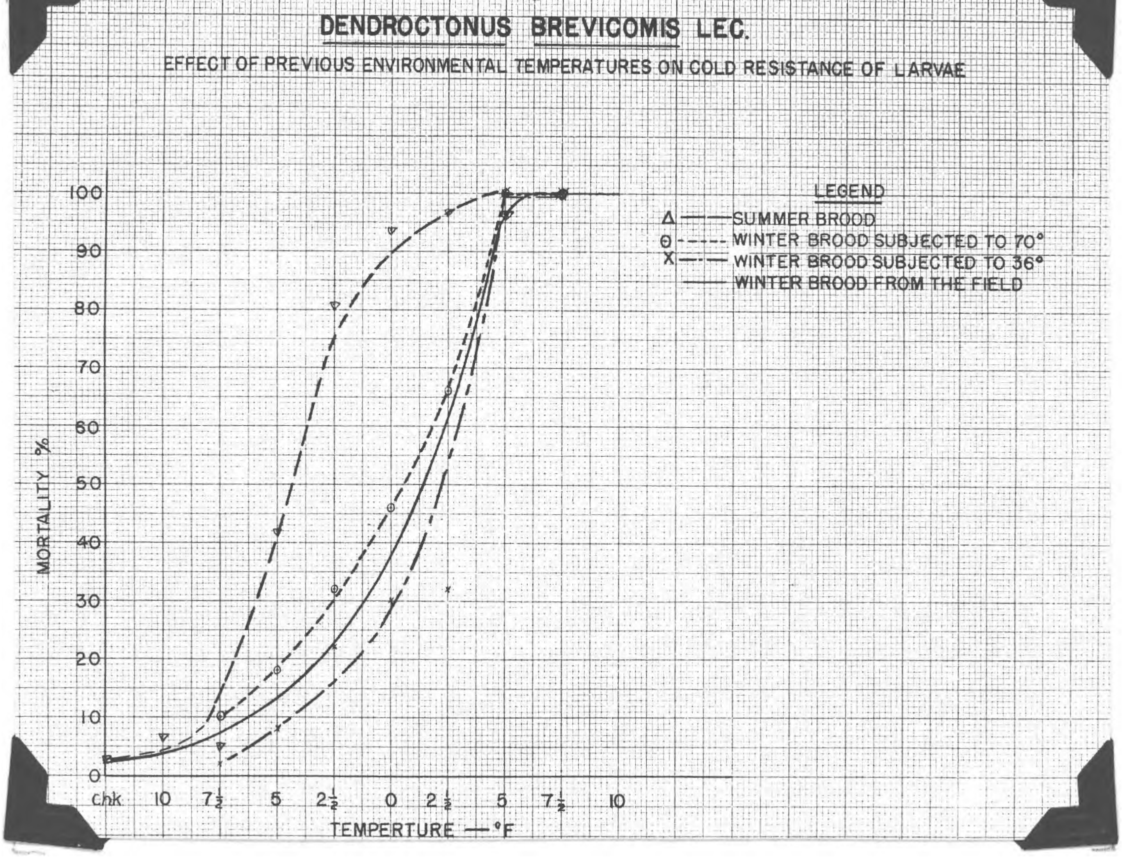
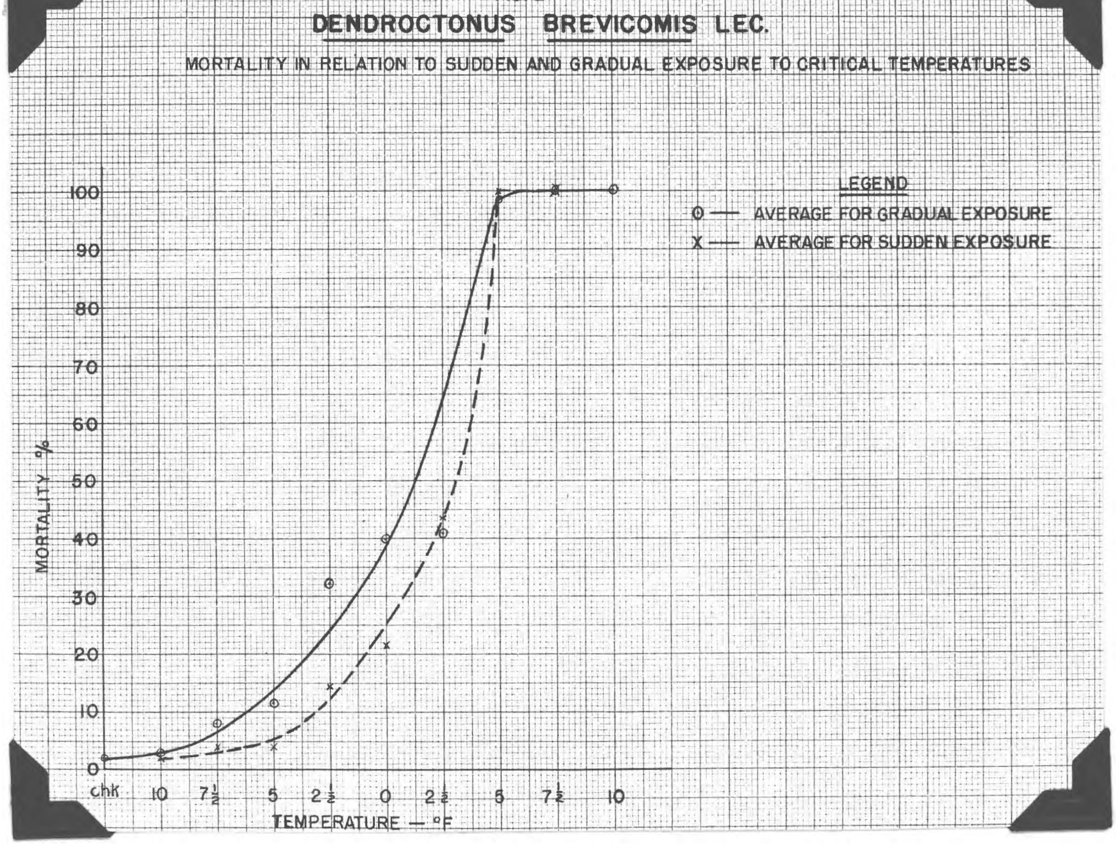

DENDROCTONUS BREVICOMIS LEC.
EFFECT OF PREVIOUS ENVIRONMENTAL TEMPERATURES ON COLD RESISTANCE OF LARVAE
LEGEND
Δ —— SUMMER BROOD
Θ ----- WINTER BROOD SUBJECTED TO 70°
X —·— WINTER BROOD SUBJECTED TO 36°
—— WINTER BROOD FROM THE FIELD
MORTALITY %
0
10
20
30
40
50
60
70
80
90
100
chk
10
7½
5
2½
0
2½
5
7½
10
TEMPERTURE — °F

FIG. 2


DENDROCTONUS BREVICOMIS LEC.
MORTALITY IN RELATION TO SUDDEN AND GRADUAL EXPOSURE TO CRITICAL TEMPERATURES
LEGEND
Θ —— AVERAGE FOR GRADUAL EXPOSURE
X —— AVERAGE FOR SUDDEN EXPOSURE
MORTALITY %
0
10
20
30
40
50
60
70
80
90
100
chk
10
7½
5
2½
0
2½
5
7½
10
TEMPERATURE — °F

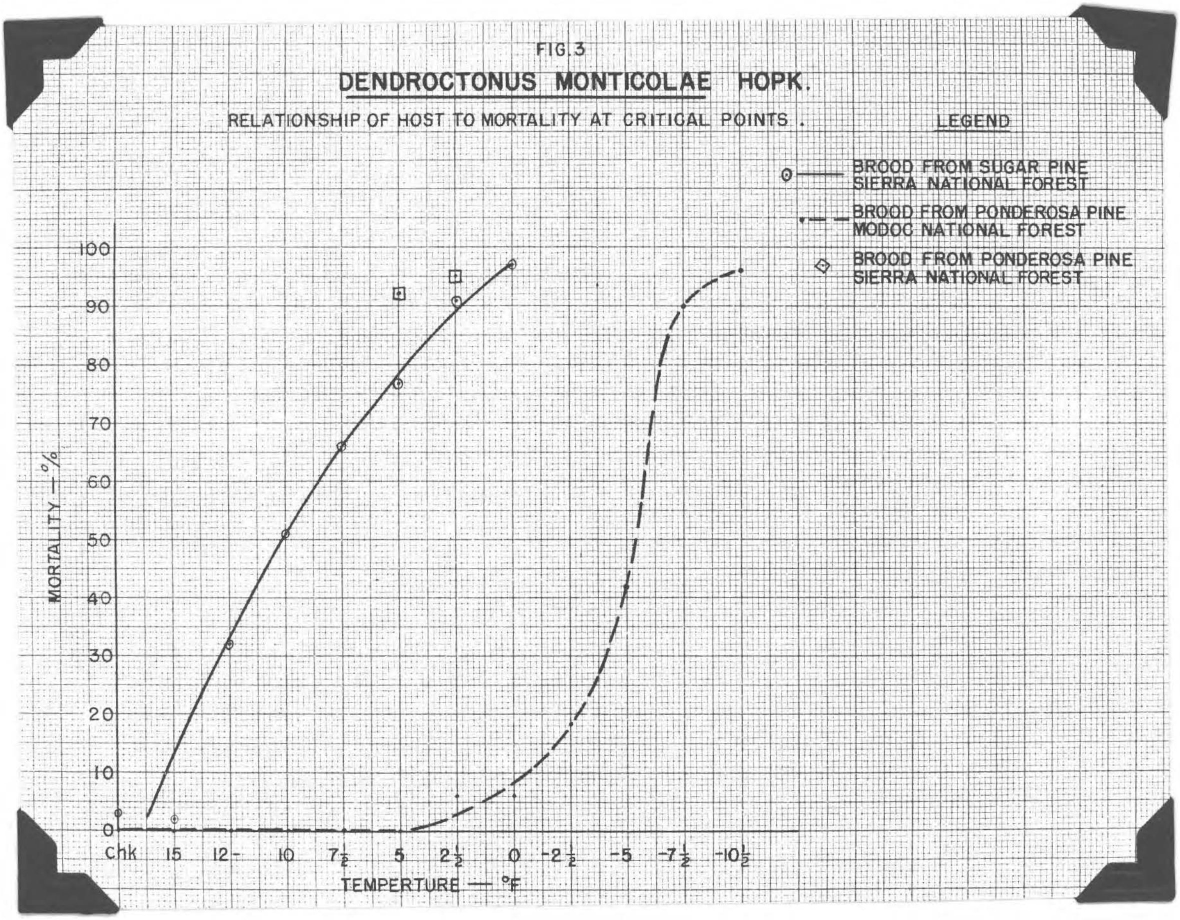


FIG. 3
DENDROCTONUS MONTICOLAE HOPK.
RELATIONSHIP OF HOST TO MORTALITY AT CRITICAL POINTS.
LEGEND
BROOD FROM SUGAR PINE SIERRA NATIONAL FOREST
BROOD FROM PONDEROSA PINE MODOC NATIONAL FOREST
BROOD FROM PONDEROSA PINE SIERRA NATIONAL FOREST
MORTALITY — %
100
90
80
70
60
50
40
30
20
10
0
Chk
15
12
10
7½
5
2½
0
-2½
-5
-7½
-10½
TEMPERTURE — °F